

## SOME PHYSICOCHEMICAL PROPERTIES OF SPECIFIC POLYSACCHARIDES\*

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In recent years the study of immunologically specific polysaccharides has not only contributed to the understanding of bacterial specificity (1) but has also proved of purely chemical interest because of the unusual structure of certain members of the group. Thus the specific polysaccharide of Type III pneumococcus has been shown to be a polyaldobionic acid built up of glycurono-glucose units (2, 3). It is, moreover, a typical colloid in the sense that it does not diffuse through collodion or parchment into water, and is, by virtue of its structure, so strong an acid that its aqueous solutions turn Congo red paper blue. Hence it seemed of interest to study some of the physicochemical properties of solutions of its salts, the more so as little is known of the behavior to be expected of an organic, colloidal, strong electrolyte devoid of basic groups. Accordingly, the sodium salt of the Type III pneumococcus specific polysaccharide was subjected to a study of its viscosity, conductance, and its behavior on diffusion. Measurements were also made on solutions of other specific polysaccharides. The diffusion data are reserved for a forthcoming paper dealing with the molecular weight of specific polysaccharides, in which connection the data recorded in the present communication will again be taken up.

Physicochemical studies have previously been made of gum arabic, a carbohydrate of a character somewhat analogous to that of the Type III pneumococcus polysaccharide. This substance, however, is the salt of a much weaker acid than the Type III

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pneumococcus carbohydrate, since the equivalent weight of arabic acid is about 1200, while the Type III substance (hereinafter referred to as S III) possesses one carboxyl group for every 340 of molecular weight. Thomas and Murray (4) have given a titration curve of arabic acid and also studied its osmotic pressure and viscosity at various hydrogen ion concentrations, and concluded that its behavior might be satisfactorily explained on the basis of the

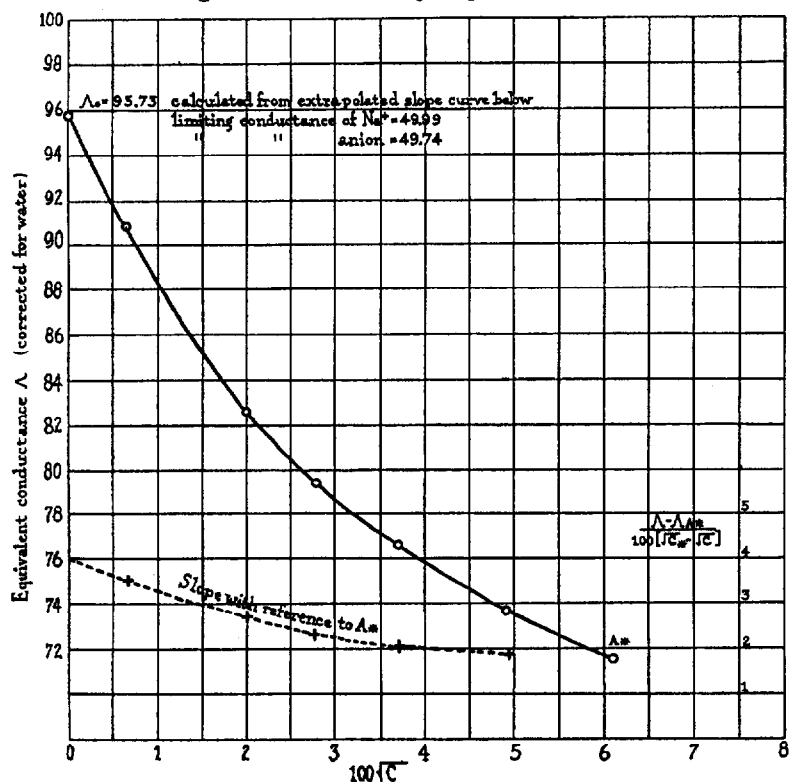


FIG. 1. Conductance of sodium salt of pneumococcus Type III specific polysaccharide.

Donnan equilibrium. The application of this conclusion to the viscosity data was rejected by Taft and Malm (5) on the basis of an extended study which included conductivity measurements. These resulted in the conclusion that the current was carried mainly by the inorganic ions present.

TABLE I  
Conductivity of Na Salt of S III at  $25^{\circ} \pm 0.00^{\circ}$

Total weight of stock solu- tion added	Equivalents $\times 10^{-3}$	Volume (at $25^{\circ}$ )	$C \times 10^3$	$100 \sqrt{C}$	Resistance reading in bridge ( $R$ )	$\frac{10^4}{R}$	$\frac{10^4}{R'}$	Uncorrected specific con- ductance ( $L'$ ) $\times 10^4$	Specific con- ductance cor- rected for water ( $L$ ) $\times 10^4$	Equivalent conductance ( $\Lambda$ )
gm.		ml.	equivalents		ohms			mhos	mhos	mhos
0	0	929.7	0	0	9917.0	1.00837	0.01018	0.005996		
1.0631	3.8862	930.7	0.041755	0.647	9321.5	1.07279	0.07460	0.043936	0.037940	90.863
10.6849	39.059	940.4	0.41534	2.04	6286.6	1.59069	0.59250	0.34895	0.34295	82.571
20.2357	73.972	949.9	0.77873	2.79	4859.0	2.05804	1.0599	0.62420	0.61820	79.385
35.9751	131.507	965.4	1.3622	3.69	3597.8	2.77948	1.7813	1.0491	1.0431	76.575
66.4993	243.088	995.6	2.4416	4.94	2460.8	4.06372	3.0655	1.8054	1.7994	73.697
105.4481	385.466	1034.2	3.7272	6.11	1805.0	5.54017	4.5420	2.6750	2.6690	71.609

Concentration of stock solution,  $3.6555 \times 10^{-3}$   $\frac{\text{equivalents}}{\text{gm. solution in air}}$ .

Equivalent weight, 340.

The cell was shunted by a resistance coil ( $S$ ) of 10018.1 ohms in the measurements.

$R$ , bridge reading.  $R'$ , actual resistance of cell.  $K$ , cell constant = 0.58895.

$$\frac{1}{R'} = \frac{1}{R} - \frac{1}{S}; L' = \frac{K}{R'}; L = L' - L_{H_2O}; \Lambda = \frac{1000 L}{C}.$$

## EXPERIMENTAL

*Conductivity Determinations*—The conductivity data were obtained with the arrangement of apparatus described by Shedlovsky (6). An S III solution was added to a new type of cell (7) from a

TABLE II  
Viscosities of S III Measured with Bingham Viscometer, No. 123,  
 $K = 1.99 \times 10^{-5}$

Temperature	Concentration	Solvent	P (corrected)	Time	$\eta$	$\eta_{\text{solvent}}$	$\eta_r$	$\eta_{sp}$	$\frac{\eta_{sp}}{C}$
°C.	per cent		gm. per sq. cm.	sec.	centi-poise	centi-poise			
20	2.93	H <sub>2</sub> O	188.90	6859	25.78	1.0050	25.65	24.65	8.41
	0.293	"	117.39	1390.6	3.248	1.0050	3.232	2.232	7.62
	0.293	"	216.4	752.4	3.234	1.0050	3.218	2.218	7.57
	0.0293	"	214.05	352.7	1.503	1.0050	1.495	0.495	16.89
	0.0293	"	99.18	799.7	1.539	1.0050	1.531	0.531	18.12
	0.0293	"	280.3	266.1	1.484	1.0050	1.477	0.477	16.28
30	0.0293	"	99.12	617.9	1.219	0.8007	1.522	0.522	17.82
	0.0293	"	208.99	286.4	1.191	0.8007	1.488	0.488	16.66
	0.0293	"	280.32	211.3	1.179	0.8007	1.472	0.472	16.11
20	0.0293	0.17 M NaCl	280.04	192.8	1.074	1.0090	1.064	0.064	2.18
	0.0293	0.17 " "	193.23	278.7	1.072	1.0090	1.062	0.062	2.12
	0.0293	0.17 " "	110.87	487.0	1.075	1.0090	1.065	0.065	2.22
30	0.0293	0.17 " "	111.08	388.3	0.858	0.809	1.061	0.061	2.08
	0.0293	0.17 " "	212.37	202.8	0.857	0.809	1.059	0.059	2.01
20	0.0586	0.17 " "	233.86	243.7	1.134	1.009	1.124	0.124	2.12
	0.0586	0.17 " "	132.31	431.4	1.134	1.009	1.124	0.124	2.12
	0.0293	0.85 " "	132.25	426.9	1.124	1.078	1.042	0.042	1.43
	0.0293	0.85 " "	166.79	338.4	1.123	1.078	1.042	0.042	1.43
	0.0293	0.85 " "	219.27	257.8	1.124	1.078	1.043	0.043	1.47

weight burette, so that increasing concentrations were measured without emptying the cell. The equivalent weight was taken as 340 and measurements were made in the range 0.039 to 3.85 milliequivalents. The extrapolation to limiting conductance was made with the aid of a derived slope curve by use of the formula given in Fig. 1. The data are recorded in Table I and Fig. 1.

That the results were not influenced by hydrolysis is indicated by

the fact that no shift in pH could be observed with either methyl red or phenol red as indicator on diluting a 30 mm solution 100 times with freshly boiled distilled water.

TABLE III  
Viscosities of *S III* Solutions. Ostwald Viscometer at 20°

Substance	Solvent	Concentration	Time	Time, solvent	$\eta_r$	$\eta_{sp}$	$\frac{\eta_{sp}}{C}$	$\frac{\eta_{sp}}{\sqrt{C}}$
		per cent	sec.	sec.				
H <sub>2</sub> O	H <sub>2</sub> O		92.6					
S III	"	0.293	303*	92.6	3.27	2.27	7.75	4.19
	"	0.0586	174.8	92.6	1.89	0.89	15.20	3.68
	"	0.0293	147.9	92.6	1.60	0.60	20.48	3.50
	"	0.0195	137.1	92.6	1.48	0.48	24.62	3.44
	"	0.01465	132.5	92.6	1.43	0.43	29.35	3.55
	"	0.01172	124.4	92.6	1.34	0.34	29.10	3.14
KCl	H <sub>2</sub> O	1.21 M	86.6					$\frac{\eta_{sp}}{C}$ corrected for volume of KCl
S III	1.21 M KCl	0.2930	129.9	86.6	1.50	0.50	1.71	1.65
	1.21 " "	0.0293	90.5	86.6	1.05	0.05	1.71	
KCl	H <sub>2</sub> O	3.35 M	82.4					0.64
S III	3.35 M KCl	0.293	99.4	82.4	1.21	0.21	0.72	
	3.35 " "	0.0293	84.3	82.4	1.02	0.02	0.68	
Na <sub>2</sub> HPO <sub>4</sub>	H <sub>2</sub> O	0.05 M	95.5					
S III	0.05 M Na <sub>2</sub> HPO <sub>4</sub>	1.00	406.7	95.5	4.26	3.26	3.26	
	0.05 " "	0.10	117.0	95.5	1.23	0.23	2.30	
HCl	H <sub>2</sub> O	0.27 M	94.1					
S III	0.27 M HCl	0.0293	97.7	94.1	1.04	0.04		
HCl	H <sub>2</sub> O	2.60 M	101.5					
S III	2.60 M HCl	0.0293	106.1	101.5	1.05	0.05		
NaCl	H <sub>2</sub> O	0.17 M	93.7					
S III	0.17 M NaCl	0.0293	99.6	93.7	1.06	0.06		

\* Corrected for relative density of solution,  $d_{20}^{20} = 1.003$ ;  $T_{obs.} \times d$ .

The conductivity determinations were made at The Rockefeller Institute for Medical Research by Dr. T. Shedlovsky, and it is a pleasure for the writers to express their indebtedness to him for his freely given time and invaluable aid in the interpretation of the

data. Thanks are also due Dr. Duncan A. MacInnes of the Rockefeller Institute for his interest and assistance.

TABLE IV  
Viscosities of *S I* and *S II* in Ostwald Viscometer at 20°

Sub- stance	Solvent	Concen- tration	Time	Time, solvent	$\eta_r$	$\eta_{sp}$	$\frac{\eta_{sp}}{C}$	$\frac{\eta_{sp}}{\sqrt{C}}$
		per cent	sec.	sec.				
H <sub>2</sub> O			92.9					
<i>S I</i>	H <sub>2</sub> O	0.3273	187.9*	92.9	2.05	1.05	3.21	1.84
	"	0.06546	122.3	92.9	1.32	0.32	4.89	1.25
	"	0.03273	108.7	92.9	1.17	0.17	5.20	0.94
	"	0.02181	105.6	92.9	1.14	0.14	6.42	0.95
	"	0.01637	103.2	92.9	1.11	0.11	6.72	0.86
		0.0131	100.5	92.9	1.08	0.08	6.11	0.70
KCl	H <sub>2</sub> O	1.34 M	86.0					$\frac{\eta_{sp}}{C}$ corrected for vol- ume of KCl
<i>S I</i>	1.34 M KCl	0.3273	116.5	86.0	1.35	0.35	1.07	1.03
	3.35 " "	0.3273	109.6	82.4†	1.33	0.33	1.01	0.90
	0.17 " NaCl	0.3273	132.8	93.7†	1.42	0.42	1.28	
	0.27 " HCl	0.3273	141.4	94.1†	1.50	0.50	1.53	
	2.60 " "	0.3273	153.0	101.5†	1.51	0.51	1.56	
	0.008 M HCl	0.3273	164.5	92.6†	1.78	0.78	2.38	
	0.009 " "	0.2975	146.2	92.6†	1.58	0.58	1.95	
H <sub>2</sub> O			93.1					
<i>S II</i>	H <sub>2</sub> O	1.106	113.7†	93.1	1.22	0.22	0.20	
	"	0.221	97.2	93.1	1.04	0.04	0.18	
NaCl	"	0.85 M	96.5					
<i>S II</i>	0.85 M NaCl	0.553	103.2	96.5	1.07	0.07	0.13	

\* Corrected for relative density of solution,  $d_{20}^{20} = 1.002$ .

† From Table III. Time for H<sub>2</sub>O is used for 0.008 and 0.009 M HCl.

‡ Corrected for relative density of solution,  $d_{20}^{20} = 1.007$ .

*Viscosity Measurements*—The viscosity data given in Table II were determined as described by Bingham (8) with the Bingham viscometer at Lafayette College, and the writers wish to thank Professor E. C. Bingham for extending to them the hospitality of his laboratory and the benefit of his personal interest. All pres-

tures were corrected for temperature, hydrostatic head, and flow. The viscosities were expressed in absolute units and the relative and specific viscosities calculated.

The remaining viscosities were determined with an Ostwald viscometer at 20°, the value obtained with an S III solution within the range of the Hagen-Poiseuille law checking closely with that found with the aid of the Bingham instrument. 5.0 cc. of solution were used in every case. Potassium chloride was used instead of sodium chloride at the higher salt concentrations since its effect on the viscosity of water is almost negligible.

TABLE V  
*Viscosities of Specific Polysaccharides in Ostwald Viscometer at 20°*

Substance	Concentration	Time	$d$	$T \times d$	Time, solvent	$\eta_r$	$\eta_{sp}$	$\frac{\eta_{sp}}{C}$
Specific gum arabic (S.G.A.)								
H <sub>2</sub> O		93.1						
S.G.A.	4.85	143.2	1.022	146.4	93.1	1.57	0.57	0.12
"	0.485	99.0	1.002	99.2	93.1	1.07	0.07	0.14
"	0.0485	94.0			93.1	1.01	0.01	0.21
Bovine tubercle bacillus polysaccharide (B.B.G. 526 I A*)								
H <sub>2</sub> O		92.6						
B.B.G.	1.218	102.1	1.003	102.4	92.6	1.11	0.11	0.09
"	0.1218	93.9			92.6	1.01	0.01	0.08

\* To be described in a later publication.

The results are given in Tables III to V and in part in Fig. 2.

*Titration of Type I Pneumococcus Specific Polysaccharide*—It will be recalled that S I is both a weak base and strong acid (9). Since the isoelectric substance dissolves only slowly in alkali the titration was carried out as follows: A weighed sample of anhydrous S I was wet with water and dissolved with a measured amount of N/14 hydrochloric acid. The solution was then titrated back through the isoelectric point with N/14 sodium hydroxide. The finely divided precipitate dissolved readily at a reaction which was still acid to litmus, and the titration was continued to alkalinity

to phenolphthalein or thymolphthalein, very little additional alkali being required between the turning points of the two indicators.

0.3153 gm. of S I 58, dissolved in 10.03 cc. of  $N/14$  HCl, required 25.23 cc. of  $N/14$  NaOH (0.15 cc. being deducted as indicator blank) to blue color to thymolphthalein, or 15.20 cc. in excess, equivalent to 1.086 cc. of  $N$  NaOH. Acid equivalent, 290.

0.4048 gm. of S I 59 B, dissolved in 10.03 cc. of  $N/14$  HCl, required 27.28 cc. of  $N/14$  NaOH to bright pink to phenolphthalein,

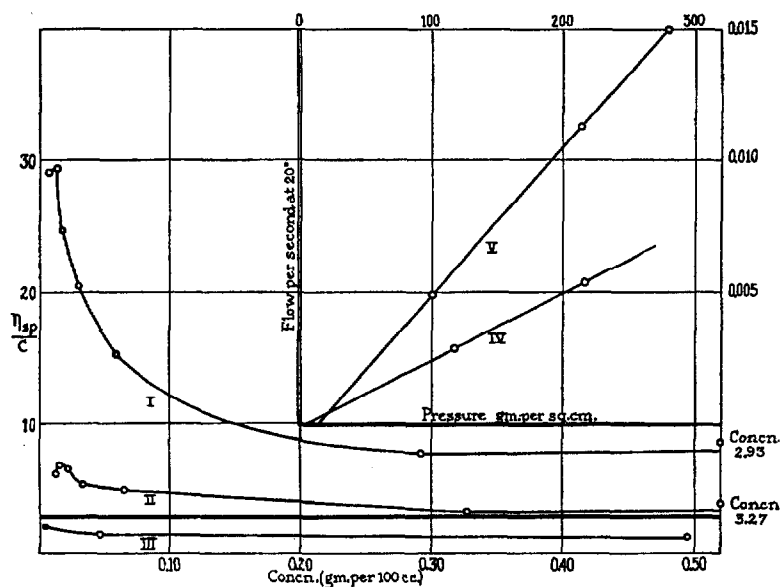


FIG. 2. Viscosity relations of sodium salts of specific polysaccharides. Curve I, S III; Curve II, S I; Curve III, S. G. A. (ordinates  $\times 0.1$ ; abscissæ  $\times 10$ ); Curves IV and V, S III 0.293 and 0.0293 per cent, respectively.

or 17.25 cc. in excess, equivalent to 1.232 cc. of  $N$  NaOH. Acid equivalent, 328. Mean acid equivalent, 309.

#### DISCUSSION

The high values of the equivalent conductance shown in Table I and Fig. 1 indicate that the sodium salt of S III is a strong electrolyte, since  $\Lambda$ , which is 95.7 at infinite dilution, drops to only 71.6 at a concentration of 3.85 milli-equivalents.



The conductivity of S III, however, does not follow the linear relationship between  $\Lambda$  and the square root of the concentration,  $\Lambda = \Lambda_{\infty} - a\sqrt{C}$ , derived empirically by Kohlrausch and given theoretical significance by Debye and Hückel and by Onsager (10). In a highly ionized substance, the slope of the conductance curve,  $a$ , depends largely on the valence type, increasing with the valence. In the case of an electrolyte which is incompletely dissociated, such as thallium chloride (*cf.* (10)), dilution decreases the slope, owing to the increase in the number of conductors, and the resulting curve is concave toward the origin. In the S III salt, however, this effect is more than counterbalanced, and the curve becomes convex toward the origin. This may be explained by assuming, with increasing dilution, the formation of ions of higher and higher valence type as additional COONa groups dissociate. The possibility of this taking place is shown by the structural formula for the S III salt given below in another connection. Thus, the slope,  $a$ , of the conductivity curve would tend to increase with dilution, rather than decrease, owing to the preponderating influence of the increasing valence. That even a simpler substance may show a similar deviation from a straight line relationship is readily demonstrated by plotting the equivalent conductance curves calculated from Noyes and Lombard's measurements for the tetra and penta sodium salts of benzene pentacarboxylic acid (11).

Since the limiting conductance of the sodium ion is 50.0, that of the Type III polycarboxylate ion (equivalent weight 340) would be  $95.7 - 50.0$ , or 45.7, indicating that it is a ready carrier of electricity. Its behavior is thus very different from that ascribed to a polysaccharide anion such as that of gum arabic (equivalent weight 1200) by Taft and Malm (5), who consider the inorganic ions responsible for the entire current carried. The value of the limiting conductance is, indeed, very close to recent values ascribed to the caseinate (equivalent weight 2000) (12) and globulinate ions (equivalent weight 3000) (13). The S III polycarboxylate ion, however, is free from basic groups and has a carboxyl group for every 340 of molecular weight. Since all of the available evidence indicates that there are at least eight to ten such groups in the molecule, the cumulative effect of the negative charges on the S III ion would be very large. That a total charge of this magnitude would result in large interionic or Coulomb forces can readily be appreciated.

Tables II and III show that although the specific viscosity,  $\eta_{sp} = \eta_r - 1$ , is independent of a  $10^\circ$  increase in temperature, indicating that there is no association or dissociation on changing the

TABLE VI  
*Specific Volumes of Dissolved Specific Polysaccharides According to Kunitz's Formula*

Substance	Solvent	Concentration	$\eta_r$	$\phi$	Specific volume	$\frac{\eta_{sp}}{C}$
		per cent		per cent	cc. per gm. solute	
S III	H <sub>2</sub> O	0.293	3.27	23.5	80	
	"	0.0586	1.89	13.4	230	
	"	0.0293	1.60	10.0	340	
	"	0.0195	1.48	8.4	430	
	"	0.01465	1.43	7.7	530	
	"	0.01172	1.34	6.3	540	
	1.21 M KCl	0.2930	1.50	8.7	30	
	1.21 " "	0.0293	1.05	1.1	38	
	3.35 " "	0.2930	1.21	4.2	14	
	3.35 " "	0.0293	1.02	0.44	15	
	0.05 " Na <sub>2</sub> HPO <sub>4</sub>	1.00	4.26	28.3	28	
	0.05 " "	0.10	1.23	4.6	46	
S I	H <sub>2</sub> O	0.3273	2.05	14.9	46	
	"	0.0327	1.17	3.5	110	
	"	0.0218	1.14	3.0	140	
	"	0.0131	1.08	1.8	140	
	1.34 M KCl	0.3273	1.35	6.5	20	
	3.35 " "	0.3273	1.33	6.2	19	
S II	H <sub>2</sub> O	1.106	1.22	4.4	4	
	"	0.221	1.04	0.87	4	
	0.85 M NaCl	0.553	1.07	1.6	3	
S.G.A.	H <sub>2</sub> O	4.85	1.57	9.6	2	
	"	0.485	1.07	1.6	3	
Na benzene pentacarboxylate (Noyes and Lombard)	"	2.04	1.09	2.0	1.0	0.044
	"	0.816	1.04	0.87	1.1	0.049
	"	0.245	1.015	0.33	1.3	0.061

temperature in this range, aqueous solutions of S III obey neither the Hagen-Poiseuille law, nor the Einstein relation,  $\eta = \eta_0 \left(1 + K \frac{N\phi}{V}\right)$ , from which  $\frac{\eta_{sp}}{C}$  should equal a constant. It is clear

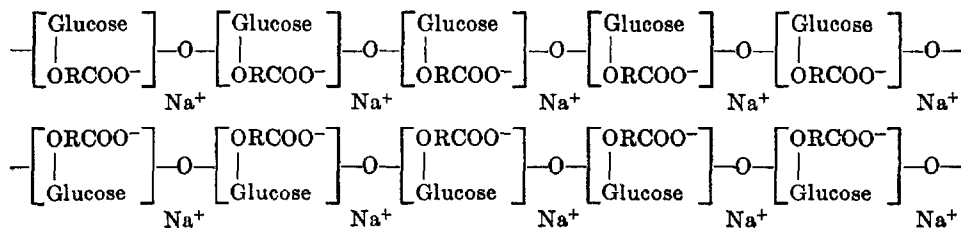
(Fig. 2) that the latter function increases to a maximum with increasing dilution, just as the equivalent conductance increases with dilution. It is, therefore, possible to ascribe the increasing deviation from the Einstein viscosity relation to the same cause; namely, the ionization of additional COONa groups and the resultant increase in the charge on the polycarboxylate ion. In this instance the relation

$\frac{\eta_{sp}}{\sqrt{C}}$  is found to hold over a limited range of concentration, but this is not so evident in the other specific polysaccharides studied. Moreover, the viscosity determinations made by Noyes and Lombard (11) on solutions of sodium benzene pentacarboxylate show a 40 per cent increase in  $\frac{\eta_{sp}}{C}$  with decreasing concentration from 2 per cent (50 mm) to 0.25 per cent (6 mm) (Table VI). Although this is small compared with the effect noted in the case of S III, the negative charge due to the piling up of carboxyl groups on even a substance of low molecular weight appears to result in a viscosity effect in the same direction.

It is also possible to ascribe the observed viscosity effects to hydration of the specific substance. If this be true, the hydration must be of a most unusual magnitude. Otherwise, at the low concentrations of S III at which  $\frac{\eta_{sp}}{C}$  is still increasing, the concentration of free water in the system would be practically constant. If hydration be the cause of the observed anomalies, it should be possible to calculate its extent by means of Kunitz's modification (14),  $\eta = \frac{1 + 0.5 \varphi}{(1 + \varphi)^4}$ , of Einstein's formula, and this does indeed lead to enormous values for the specific volume in dilute aqueous solution (Table VI), 1 gm. of S III being capable, according to the formula, of combining with as much as 540 gm. of water.

It is also shown in Fig. 2 that if the rate of flow per second of dilute solutions of S III in water is plotted against the pressure, a straight line is obtained for each concentration. These lines do not pass through the origin, but intersect the axis at a positive value for  $P$  which increases with dilution. Thus a certain pressure must be exceeded before flow starts, and the solution behaves as a plastic solid. This might be accounted for by assuming a definite orientation of the highly charged polycarboxylate ion at high dilutions

at which dissociation is approaching completion. This would also entail an orientation of the accompanying sodium ions, and a definite structure would result, somewhat as follows:



in which  $\text{RCOO}^-$  represents the glycuronic acid residue in glucosidic union with the glucose residues (*cf.* (3)). Such an arrangement should also result in a maximum value of  $\frac{\eta_{sp}}{C}$  and is consistent with the explanation given for the positive deviation from the square root conductivity relation at high dilutions.

In the case of the specific polysaccharide of Type I pneumococcus the viscosity anomalies are smaller than in the case of S III, and this might be ascribed either to the smaller magnitude of the Coulomb forces engendered by the S I anion, or to the lower extent of the hydration. The acid equivalent of S I is somewhat lower than that of S III, but since there is one basic group for approximately every three carboxyl groups, the total negative charge on the S I ion would be less than that of S III, assuming the molecular weights to be approximately the same. Also, calculation of the hydration by Kunitz's formula yields lower values than in the case of S III, so that either explanation might be valid.

In the case of S II and the specific gum arabic, with their acid equivalents of approximately 1000, both viscosity and hydration are low. Since there is an apparent parallel between the total acidity of the specific polysaccharides considered and their hydration as calculated by the Kunitz formula, it is possible that the unusual hydration, or at least the unusual viscosity, of the S III and S I is conditioned by the unusual magnitude of the total negative charge on these ions.

Association of abnormally high viscosities with an acid polysaccharide has been shown qualitatively by Raistrick and Rintoul (15) in the case of luteic acid, a polysaccharide produced from

glucose by *Penicillium luteum*, Zukal. The structural unit is composed of 2 molecules of glucose and 1 of malonic acid, one of the carboxyl groups being free. Moreover, Staudinger and Kohlschütter (16) have recently continued earlier work by Staudinger and Urech (17), giving preliminary viscosity data on polyacrylic acids. They fix the relative viscosity of a 0.1 equivalent solution of a sodium salt at the enormous value of 114 and the relation  $\frac{\eta_{sp}}{C}$  at about 1140, values far higher than

obtained in the case of S III. The polyacrylic salts differ from those of S III, however, in hydrolyzing readily and in the smaller equivalent conductivity of their aqueous solutions. Staudinger originally considered the high viscosity of polyacrylic acid salts to be due to hydration caused by the high ionic charge, but now ascribes it to "swarms" caused by increasing ionization. If, as seems agreed, these high viscosities are conditioned by the piling up of large Coulomb forces, regardless of the mechanism by which this is brought about, a substance such as acrylic acid, with an acid equivalent of only 86 would be expected to develop higher viscosities as the chain of carbon atoms and carboxyl groups is lengthened by polymerization, than would the aldobionic acid structural unit of S III, with its acid equivalent of 340.

Tables II and III show clearly the effect of electrolytes in diminishing the viscosity (and in Table VI the hydration) of the solutions of S III and in bringing them into agreement with the Hagen-Poiseuille law and the Einstein equation.

It is shown in Table IV that  $\frac{\eta_{sp}}{C}$  increases to a maximum with increasing dilution in the case of the sodium salt of S I as in the case of S III, but that the increase is much smaller, and that  $\frac{\eta_{sp}}{\sqrt{C}}$  is constant over a very limited range. Although titration shows that the acid equivalent of S I is lower than that of S III, the greater number of carboxyl groups in the molecule and their expected effect on the total charge and viscosity appear to be more than negated by the simultaneous presence of basic groups. However, the internal compensation of Coulomb forces is insufficient to prevent the building up of a negative charge large enough to produce viscosity effects qualitatively similar to those encoun-

tered in the case of S III (see also Fig. 2); and in S I, also, these forces are only incompletely reduced by isotonic concentrations of salts. The smaller viscosity effects might also be attributed to a lower molecular weight for S I than for S III, but no evidence is available on this point. Although the total viscosity effects of the interionic forces due to the S I anion do not reach the proportions shown by the S III ion, when these forces are depressed by large concentrations of salts or acids, the specific viscosity of S I is very close to that of S III. This is perhaps a reflection of similarities in the arrangement of the sugar and sugar acid units of which the two substances are composed.

The low acid equivalent of S I may be taken as evidence that some other explanation must be sought for the widely differing ratios between precipitin index and mouse protection in the case of Type I and Type III antisera than the suggestion of Sobotka and Friedlander (18) that the ratios are connected with differences in the acid equivalents of the two polysaccharides.

Table V shows that in the non-basic specific gum arabic (19) (S.G.A., acid equivalent 835) the accumulation of negative charges is still great enough to give rise to an increase in  $\frac{\eta_{sp}}{C}$  on dilution, while this effect is lost in the case of the specific polysaccharide of Type II pneumococcus (9) (S II, acid equivalent 1250). The specific viscosities of these polysaccharides are also lower than those found at similar concentrations of S I and S III, but are higher than that of a non-acidic (and non-basic) bovine tubercle bacillus polysaccharide (B.B.G., Table V).

In the series of specific polysaccharides studied, therefore, there appears to be a relation between the magnitude of the viscosity phenomena shown by the substance and the frequency of occurrence of carboxyl groups in the polysaccharide chain. That the large viscosity effects exhibited by salts of S III are due to the consequent piling up of negative charges in the salts of this strong multivalent acid is believed to be indicated by the data presented.

#### SUMMARY

1. The specific polysaccharide of Type III pneumococcus is shown to yield a highly ionized sodium salt characterized by a mobile negative ion of very high valence.

2. Viscosities have been determined for a number of specific polysaccharides under varying conditions of salt concentration, and the findings correlated with the magnitude of the charge on the anion.

3. So strong are the interionic, or Coulomb, forces engendered by the S III polycarboxylate ion that the ratio  $\frac{\eta_{sp}}{C}$  increases many times with dilution. At increasing dilutions S III solutions show in increasing measure the yield point phenomenon characteristic of plastic solids, an effect possibly due to the orientation of ions brought about by these forces.

4. The specific polysaccharide of Type I pneumococcus shows viscosity abnormalities of the same character, but of smaller magnitude. Although its acid equivalent is even lower than that of S III, internal compensation of negative charges by the basic groups present is believed to be the cause of the smaller effect.

5. The data on the specific gum arabic and Type II pneumococcus polysaccharide show that the viscosity effects decrease with the relative number of carboxyl groups, or negative charges, in the molecule.

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